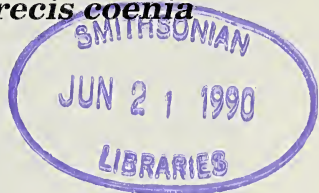


Development of the Wing Margin in *Precis coenia* (Lepidoptera: Nymphalidae)

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Abstract. The shape of the wings of Lepidoptera is determined in the larval imaginal disk by the position of a peripheral "bordering lacuna". The portion of the imaginal disk proximal to this lacuna (the wing epithelium) will form the wing proper, while cells distal to this lacuna (the peripheral epithelium) undergo programmed cell death during the pupal stage. In *Precis coenia*, cell death in the peripheral epithelium begins on the ventral side by six hours after pupation and gradually spreads throughout the epithelium over the next 72 hours. After this period of cell death the adult wing has achieved its final form and size. The most peripheral of the scale-forming cells on the adult wing become enlarged between 48 and 72 hours after pupation. These scale cells will produce the fringe of long marginal scales. Transplant experiments show that determination of these marginal scales must have occurred prior to pupation, and thus well prior to the period of cell death in the peripheral epithelium. We found that in *P. coenia* the marginal scales do not form a discrete size group but rather are the extremes of a gradient in scale size that extends in from the wing margin for at least 3 scale-cell rows. We postulate that some special property of the wing margin, presumably originating from the bordering lacuna but decaying with distance, is responsible for inducing the formation of the unusually large scales that form the marginal fringe.

Introduction

The wings of butterflies and moths develop during the larval stage as internal imaginal disks. The wing disks undergo a substantial amount of morphological differentiation during late larval life, so that by the middle of the last larval instar they are usually readily identifiable as miniature wings complete with a primitive venation pattern (Nijhout, 1985). The wing veins develop initially as a system of lacunae between the dorsal and ventral epidermal layers of the wing disk. These lacunae radiate out from the base of the wing disk in a branching pattern that presages the future wing venation. In addition, a peripheral bordering lacuna develops, that runs roughly parallel to the margin of the disk. Suffert (1929) showed that this bordering lacuna marks the position of the future margin of the adult wing. During the pupal stage all cells

peripheral to the bordering lacuna disappear and only the portion of the imaginal disk within the periphery of the bordering lacuna will become the wing of the adult. As a consequence, the size and shape of the adult wing is determined by the position and path of the bordering lacuna within the wing imaginal disk. The fine features of the wing shape, such as tails in papilionids and saturniids, as well as the gross morphology of the wing are determined by the path of the bordering lacuna (Suffert, 1929), and any change in wing shape, whether developmental or evolutionary, must have its basis in an alteration of the shape of the bordering lacuna.

Thus we can think of the adult wing shape as being produced by a cookie-cutter-like mechanism that outlines the precise form of the adult wing within the much larger wing imaginal disk. The margin of the adult wing is not the margin of the imaginal disk, and this has several implications when we think about structure and function at the wing margin. It makes us wonder about the morphology of the adult wing edge; about whether and how the dorsal and ventral wing surfaces become reattached at their periphery after death of the tissue distal to the bordering lacuna. It draws our attention to the fact that the margin of the adult wing is further "specialized" in that it bears a distinctive fringe of marginal scales. In most species these marginal scales are much larger, and very different in shape and color from the scales that deck the rest of the wing surface. Thus scale morphogenesis must be under a different type of control at the wing margin than elsewhere on the wing. Moreover, the wing margin appears to have an additional functional specialization. It is clear that the margin is involved in the determination of several elements of the wing's color pattern (Nijhout and Grunert, 1988), and the bordering lacuna provides an obvious structural feature that could be the source of the requisite inductive signal(s).

The present paper reports on a morphological study of cell death at the wing margin of the Buckeye butterfly, *Precis coenia*. We document the spatial and temporal pattern of cell death, the structure of the new wing margin, and the fact that the specialized fringe of marginal scales appears to be induced by a special property of the bordering lacuna at sometime prior to pupation.

Materials and Methods

Larvae of *Precis coenia* were reared at a constant temperature of 27 degrees Celsius on an artificial diet as described by Nijhout (1980a). Microscopy was done on material embedded in JB-4 Resin (Polysciences), sectioned at a thickness of 1 μ m, and stained with Lee's methylene blue-basic fuchsin (Polysciences). Cell death was determined by uptake of trypan blue according to the method of Humason (1979).

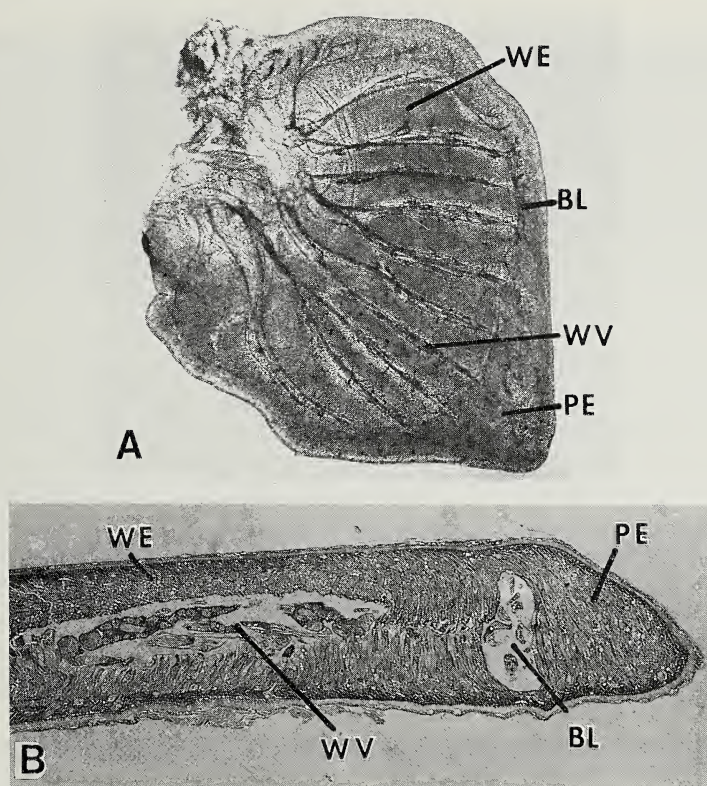


Fig. 1. A. Wholemout of imaginal disk of forewing of *Precis coenia* at day 5 of the last larval instar, and about 2 days prior to pupation. B. Crossection of distal portion of wing imaginal disk at day 5 of the last larval instar. BL, bordering lacuna; WV, lacuna of wing vein; PE, peripheral epithelium; WE, wing epithelium. Scale bar is 0.5 mm.

Specimens for scanning electron microscopy (SEM) were air-dried, coated with gold-palladium, and examined and photographed with a JEOL T20 electron microscope.

Results

At the time of pupation the wing has two surfaces each of which is an epithelial cell layer of columnar, tightly packed cells. These surfaces meet and are continuous at the periphery so that the wing's structure resembles a flat bag. The shape of the adult wing is marked by a peripheral lacuna (Fig. 1) which runs roughly parallel to the margin of the disk (Nijhout, 1985). The results presented below will show that this bordering lacuna divides the wing surface into a "distal epithelium" which is located distal to the lacuna and which will die during development, and a "wing epithelium" which will form the adult wing (Fig. 1).

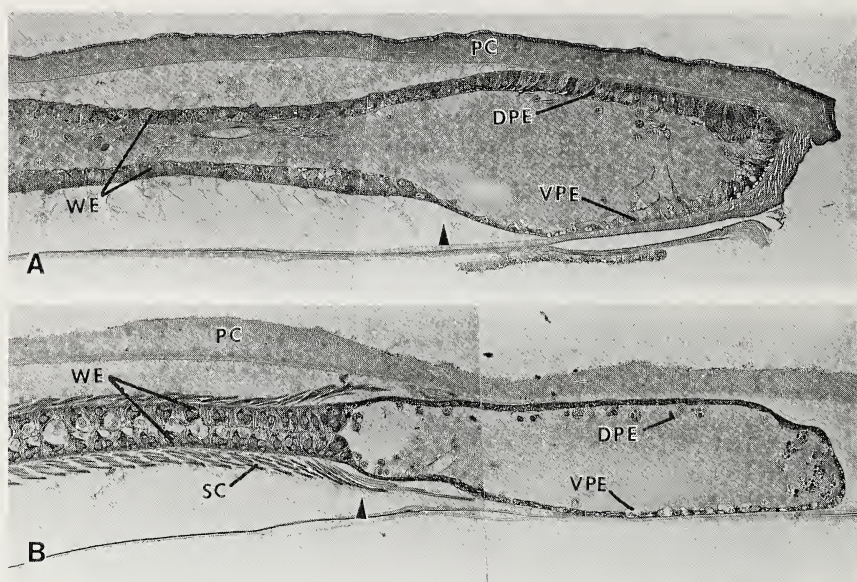


Fig. 2. A. Cross-section of pupal wing at 48 hours after pupation. B. Cross-section of pupal wing at 72 hours after pupation. Former position of bordering lacuna is shown by arrow. *PC*, pupal cuticle; *SC*, scales of the adult wing; *VPE*, ventral peripheral epithelium; *DPE*, dorsal peripheral epithelium; *WE*, wing epithelium.

Six hours after pupation the dorsal and ventral epithelial cell layers of the pupal wing had separated and surrounded a space filled with hemolymph and free-floating hemocytes. Although the bordering lacuna was no longer discernable, due to the separation of the two cell layers, its former position was still marked by the fact that the cells formerly distal to the lacuna, the distal epithelium, were slightly smaller and thinner than those of the wing epithelium. The boundary between these two cell populations formed a distinct "line" in the wing epidermis. Further studies showed that this line now defined the position of the future wing edge. Trypan blue staining revealed that at this time cells in the ventral part of the distal epithelium had already started to die. Cell death began along the former position of the lacuna and spread distally into the ventral distal epithelium. Cell thickness and cell density in the ventral distal epithelium decreased gradually over the next 48 hours.

At 48 hours after pupation cell death had progressed to the dorsal part of the distal epithelium. At this time the hemolymph space within the pupal wing was gradually diminishing in volume and the dorsal and ventral epithelia in the proximal region of the wing had already become fused (Fig. 2A). At 72 hours after pupation the dorsal and ventral epithelia of the wing-proper were once more tightly apposed, and scales

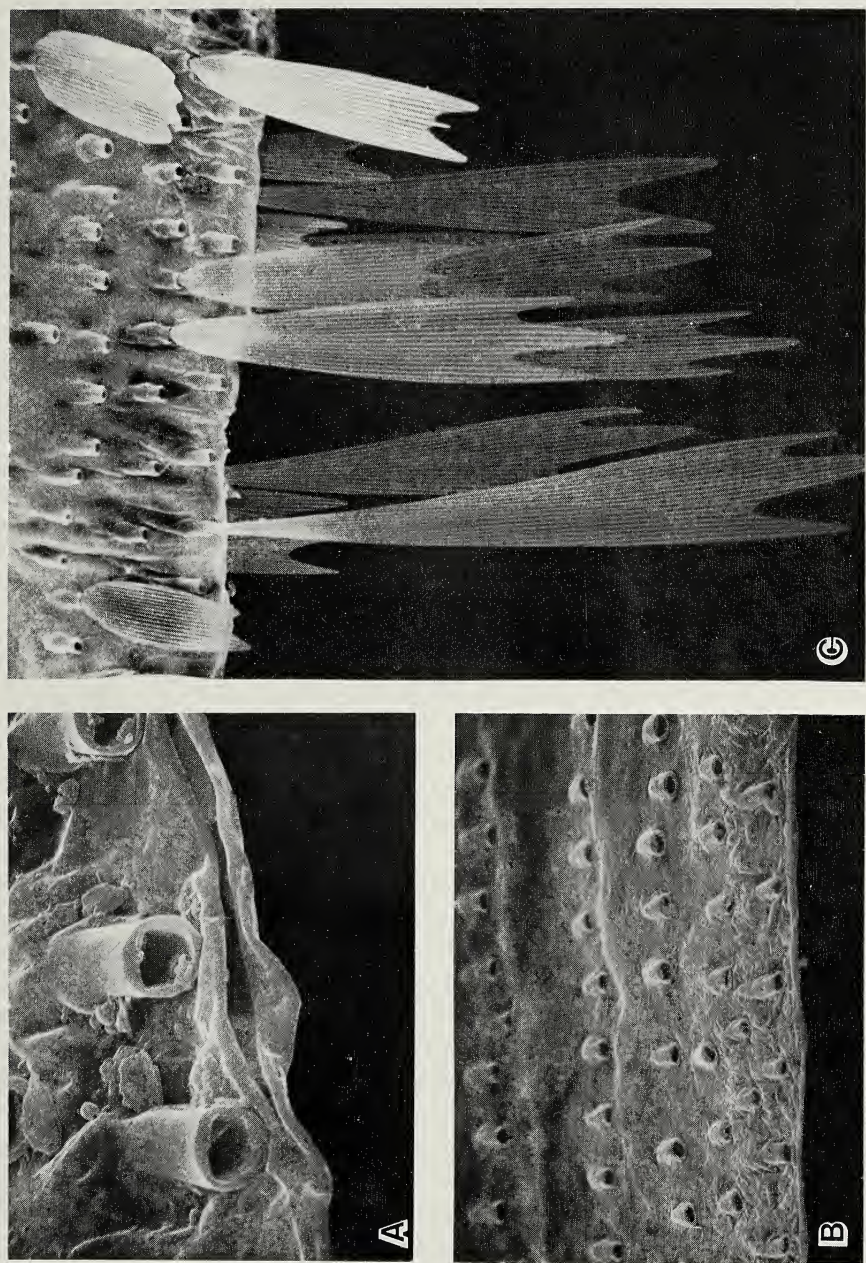


Fig. 3. Scanning electron micrographs of wing margin of adult wing. A. Edge-on view showing separation of dorsal and ventral cuticles. B. View of edge showing parallel rows of sockets of scale cells. C. View of wing margin, with a portion of scales removed, showing gradual increase in scale length with proximity to wing margin.

were in the process of formation (Nijhout, 1980b). In contrast, the dorsal and ventral parts of the distal epithelium did not become fused but remained as a thin bag-like rim around the wing (Fig. 2B). Furthermore, no enlarged scale forming cells were detectable among the surviving cell population in this peripheral epidermis, and no scales were formed in this part of the wing. The scale-forming cells at the very margin of the wing were slightly larger, and had slightly larger nuclei than those of the generalized wing epidermis. This specialization is in accord with the presumptive relation between ploidy level and scale size (Henke and Pohley, 1952).

Since the peripheral tissue of the imaginal disk undergoes cell death, no closure exists between the dorsal and ventral epidermis at the periphery of the developing adult wing. We investigated the morphology of the adult wing margin by scanning electron microscopy, and found that the dorsal and ventral cuticles never fuse but become simply appressed. The absence of fusion between the dorsal and ventral cuticles is shown by the presence of a split which separates both surfaces and which runs at or very near the wing margin Fig. 3A).

As Fig. 3B shows, the scale forming cells are arranged in straight parallel rows which take their course roughly parallel to the wing margin. We found that contrary to initial expectations there was no uniquely differentiated fringe of marginal scales. Instead the size of the scales, and the degree of indentation of their apical margin, increased gradually with proximity to the wing margin (Fig. 3C). Scales which were close to the margin were much larger than scales further inside the wing. Figure 4 shows that the size of the scales decreases exponentially with distance from the margin.

In order to determine whether cells of the peripheral epithelium were already programmed to undergo cell death at the time of pupation, we excised strips of epithelium extending across the bordering lacuna and thus consisting of both distal and wing epithelium from pupae two hours after pupation. These strips were rotated 180° and grafted back in their original site. Thus in these grafts cells of the peripheral epithelium were now placed within the wing epithelium and vice versa. The results of such a graft are shown in Fig. 5. We found that the cells of the grafted tissue always developed according to their original fate. Cells of the peripheral epithelium underwent normal cell death, occasionally leaving a hole in the wing epithelium at the site they were grafted. Most frequently, however, the epithelium contracted around the wound site, as in Fig. 5, and no hole was evident in the area where the peripheral epithelium died. Cells of the wing epithelium survived and when placed so that they could establish continuity with the rest of the wing epithelium they were retained in the adult wing. These grafts retained their original polarity as indicated by the orientation of the scales (Fig. 5). Furthermore, large marginal scales were formed at the edge of the grafted wing epithelium, extending into the space left in the wing by death of the transplanted peripheral epithelial cells.

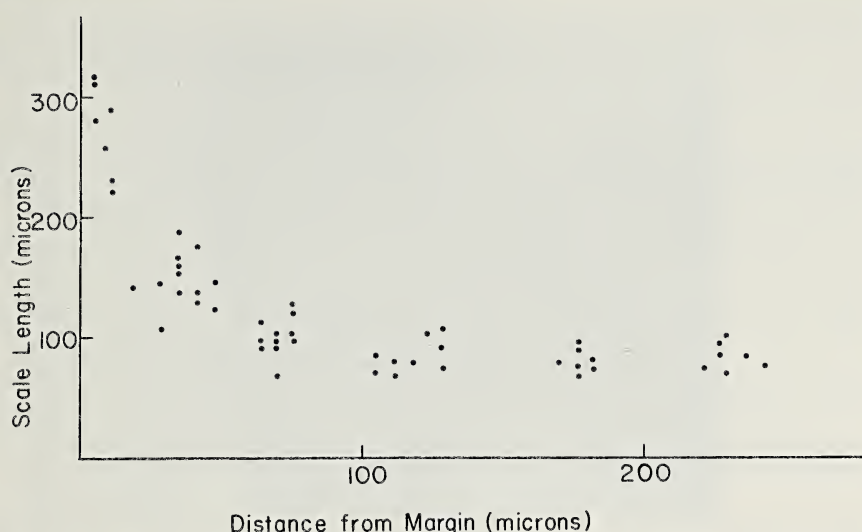


Fig. 4. Graph of scale length as a function of proximity to wing margin. Position of each scale was measured from the the aperture of its socket to the margin.

Discussion

The development of the adult wing of *Precis coenia* is a process with striking morphological changes during pupal stage. As illustrated by Figs.1 and 2 the wing starts as a flat bag-like shape. At the time of pupation it goes through a stage at which the dorsal and ventral cell layers become separated by a voluminous hemolymph-filled space. This separation coincides with the peak of mitotic activity and the later stages of color pattern determination in the wing epidermis (Nijhout, 1980b; Nijhout and Grunert, 1988). After the end of the mitotic period, about 36-48 hr after pupation (Nijhout, 1980b), the dorsal and ventral wing epithelia become closely appressed again but the distal epithelia never fuse. Instead, they form a fluid filled bag-like rim around the entire wing disk (Fig. 2B). Cell death in this peripheral epithelium begins by 6 hours after pupation and continues until at 96 h after pupation the bag-like rim has completely vanished. Thus it is not until the 4th day after pupation that the shape of the adult wing is evident.

Grafting experiments (Fig. 5) show that at two hours after pupation the cells of the distal epithelium are already programmed to die in the course of the pupal stage. The polarity of the scale forming cells has also been determined by this time, as was previously shown by Nijhout (1980a).

Determination of the elongated marginal scales has also occurred by two hours after pupation, since these scales developed normally on transplanted wing margins. Thus determination of the characteristics of the wing margin, including its position, fate of the peripheral

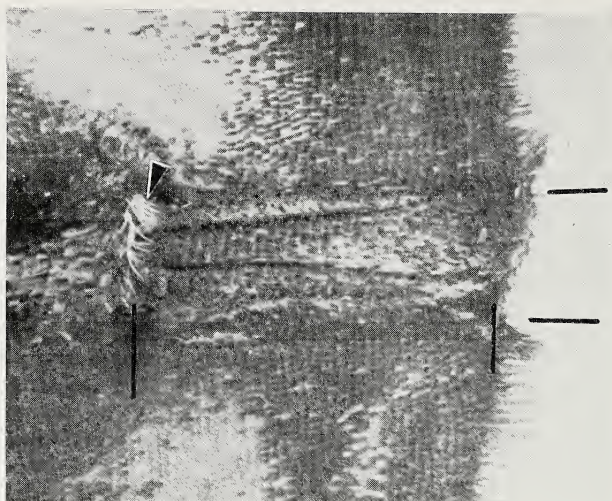


Fig. 5. Result of a grafting experiment in which a strip of pupal epidermis was excised, rotated 180 degrees, and reimplanted so that the former wing margin was now located well within the field of the wing proper. Lines at right and bottom show the limits of the graft. Arrow indicates the position of long marginal scales at the edge of the graft. In this specimen the wing epidermis has contracted and closed off the 'hole' left by the degenerated peripheral epidermis.

epithelium, and character of the marginal scales must have taken place prior to pupation. The fact that cell death in the peripheral epithelium begins at the former location of the bordering lacuna, as well as the existence of the gradient of scale size in the wing epithelium, suggest that the bordering lacuna not only serves as a demarcation between wing and peripheral epithelium but may also have an active role in inducing these two modes of cell differentiation.

The exponential increase in size of the marginal scales with proximity to the margin strongly suggests that a gradient of some sort is a determining factor. The transition from normal scales to very long marginal scales occurs over the course of 3 scale rows, or a distance of about 80 μm on the adult wing. But the adult wing of *P. coenia* is expanded by a factor of about 2 from the size of the wing in the pupal stage; thus the actual distance over which marginal cell determination gradient extends on the pupal wing is approximately 40 μm . These findings add to the body of evidence that the bordering lacuna in the wings disks of Lepidoptera plays specific and important roles in the development of the wing. Not only does it control the ultimate size and shape of the wing by outlining the areas that will undergo programmed cell death, but it also appears to possess special properties that are involved in the induction of certain elements of the color pattern (Nijhout and Grunert, 1988) and in the induction of marginal scale differentiation.

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